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Pied Flycatchers *Ficedula hypoleuca* prefer ectoparasite-free nest sites when old nest material is present

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Abstract. Nest-dwelling ectoparasitic arthropods may have detrimental effects on avian breeding success and fitness. Birds should therefore be selected to avoid nest sites where the risk of being infested by ectoparasites is high. However, studies testing this hypothesis have produced mixed results. We performed an experiment in south Norway to test whether Pied Flycatchers *Ficedula hypoleuca* select nest site according to the presence of ectoparasites. In this experiment we used artificial nest boxes which had all been successfully used by conspecifics in the previous breeding season and still contained the old nest material. Five different groups of ectoparasites were recorded in flycatcher nests in the study area, of which the haematophagous hen flea *Ceratophyllus gallinae* and mites (*Dermanyssus* sp.) occurred in all examined nests. We offered each flycatcher pair ($n = 13$) a choice between 1) a box where ectoparasites had been removed by insecticide fumigation and 2) a box in which the old nest had not been fumigated. Flycatchers were highly selective in their choice of nest site, all but one settling in the fumigated, parasite-free nest box. This finding differs from a similar Swedish study (Olsson & Allander 1995) which did not find any evidence that Pied Flycatchers avoided nest boxes with ectoparasites. A likely explanation for this discrepancy is between-population differences in parasite abundance, as the mean number of hen fleas per infested nest was about five times higher in Norway than in Sweden.

Key words: *Dermanyssus* sp.; hen fleas; nest box; parasites

INTRODUCTION

Nest-dwelling haematophagous ectoparasites include blood-sucking fleas (Siphonaptera), flies (Diptera), and mites (Acarina; Remeš & Krist 2005, López-Rull & Garcia 2015) which may negatively affect breeding success, quality of offspring and condition of parent birds (e.g. Møller et al. 1990, Loye & Carroll 1998, Fitze et al. 2004). Bird species building nests in holes and cavities (secondary cavity-nesters) where nests may remain from previous breeding events are especially exposed to nest-dwelling ectoparasites that overwinter in the old nest material (Rendell & Verbeek 1996). Such birds often breed in nest boxes, making them ideal for studies of bird-parasite interactions.

Avian nest sites vary in quality for a variety of reasons (e.g. Martin 1993, 1995, Larison et al. 1998, D'Alba et al. 2011) and birds should be selected to choose the sites where their reproductive success is least compromised. Due to the negative effects of ectoparasites, it would therefore be adaptive for secondary cavity-nesters to discriminate against nest sites with high infestation risks. The presence of old nests could for instance signal presence of ectoparasites, and earlier used nest cavities may therefore not be a preferred nest site. However, studies exploring this idea have produced mixed results. Of 24 studies (12 species) relating nest

site selection to the presence of old nests, most ($n = 15$) showed that birds acted indifferently to the presence or absence of old nest material, five studies reported preference for nest sites with old nests and only four studies reported the predicted avoidance of such nest sites (Mazgajski 2007).

A problem with the experimental procedure outlined above is that old nest material may act as a cue to nest site qualities other than the risk of ectoparasite infestation and which should in fact lead to a positive selection for earlier used nest sites. For instance, the presence of an old nest might indicate that the site was previously used in a successful breeding event, and thus is a site with low predation risk (Erckmann et al. 1990, Olsson & Allander 1995). Old nest material may also reduce the time costs of nest building (Loukola et al. 2014). Experiments manipulating the occurrence of ectoparasites directly have shown that Cliff Swallows *Hirundo pyrrhonota* (Brown & Brown 1986) and Great Tits *Parus major* (Oppliger et al. 1994, Rytönen et al. 1998) avoided infested nest sites. However, this was not the case in a study of Pied Flycatchers *Ficedula hypoleuca* and three tit species *Parus* spp. (including the Great Tit; Olsson & Allander 1995).

Due to the inconsistent results of experiments testing whether secondary cavity nesters choose nest sites in relation to the presence of ectoparasites, more

studies of this behavioural aspect would be valuable. The Pied Flycatcher is a common passerine breeding bird in the Western Palearctic that typically nests in tree holes or artificial nest boxes (Cramp & Perrins 1993, Lundberg & Alatalo 1992). The aim of this study was to test if Pied Flycatchers discriminate between parasite-infested and parasite-free old nests, to some extent repeating the experiment of Olsson and Allander (1995).

MATERIAL AND METHODS

We carried out the study at Hornnes, Aust-Agder county, south Norway (58.40° N, 7.50° E) during April–June in 1995 and 1996. Here Pied Flycatchers were common breeders in artificial nest boxes placed in a forest dominated by pine *Pinus sylvestris* and some spruce *Picea abies* interspersed with some deciduous trees, particularly birch *Betula pubescens*. Nest boxes were constructed in a standard way with inner dimensions 8.5 x 13.0 cm, while the length of the front wall was 27.0 cm and length of the back wall 28.5 cm. The diameter of the entrance hole was 32 mm. All boxes had been previously occupied by birds (Pied Flycatchers or Great Tits), and most had been out for about 13 breeding seasons. Old nest material was routinely removed from the nest boxes during the autumn and winter months.

We carried out a nest site selection experiment in spring 1996 to investigate whether Pied Flycatchers prefer parasite-free nest sites when given a choice. In this experiment, we used nest boxes which had all been successfully used by breeding flycatchers in 1995 and still contained the nest from this prior breeding event. Within randomly chosen territories occupied in 1995, we offered the flycatchers a choice between a pair of nest boxes placed in separate trees approx. 4–5 m apart. Nest boxes were paired in a random manner, and one randomly chosen nest box in each pair was translocated from another territory while the other remained in place. Nest boxes were attached to a tree with the opening facing south, about 1.5–2.0 m above

ground. In total there were 14 pairs of boxes spread out at distances of 100–150 m in the study area. To avoid interference from tits breeding earlier in spring than flycatchers, we blocked the entrance of all nest boxes and did not open them until the first days of May 1996 when Pied Flycatchers started arriving at their breeding sites. We defined a nest box as occupied if a nest was built and eggs were laid.

One randomly selected nest box in each experimental pair served as an unmanipulated control. In the other box, the inner walls and the old nest material were lightly sprayed with a 0.47% pyrethrin solution which is poisonous to invertebrates (Pillmore 1973). This treatment took place on 7–8 April 1996, i.e. about three weeks before the first flycatchers arrived. Pyrethrin is extracted from plants in the Chrysanthemum family and has been frequently used as an insecticide in similar experiments (Møller 1990, de Lope *et al.* 1993, Olsson & Allander 1995, Dufva & Allander 1996, Bauchau 1997, Tomás *et al.* 2007). Although pyrethrin solutions are not known to negatively affect adult birds, they might have some effects on nestlings (Hund *et al.* 2015). However, we assume that our experimental treatment had small effects on the welfare of nestlings because we treated experimental nests several weeks before eggs hatched, and the pyrethrin most likely degraded considerably during this period. Moreover, we found no differences in fledging success and chick growth between control and fumigated nest boxes sprayed two days after hatching in a separate study in 1995 (A. Breistøl, unpubl. data).

Since we wanted the nests in experimental boxes to be as natural as possible, it was not possible to count the ectoparasites in them directly to get an idea of the infestation risk. However, in a sample of nests in 1995 we estimated the prevalence (% of nests infested by a given ectoparasite) and intensity (mean number of parasites per infested nest) within the study population. In 15 nest boxes we counted Diptera (louse flies [Hippoboscidae] and blow flies *Trypocalliphora braueri*) during the nestling period. To extract other nest-dwelling arthropods, we collected eight nests on the day of fledging or the day after and stored these

Table 1. Prevalence (% of nests infected) and mean intensity (mean number of parasites per infested nest) \pm SE of different ectoparasites in random samples of Pied Flycatcher nests at Hornnes, Aust-Agder county, south Norway in 1995. Louse flies and blow flies were counted during the nestling phase, while other animal groups were extracted by using Berlese-Tullgren funnels (see methods). No parasites were found in fumigated nests ($n = 11$).

Type of ectoparasite	n	Prevalence	Mean intensity	Min.	Max.
Lice (Mallophaga)	8	50	779 \pm 1101	1	6229
Fleas (<i>Ceratophyllus gallinae</i>)	8	100	51 \pm 14	4	122
Louse flies (Hippoboscidae)	15	60	1.9 \pm 0.3	1	3
Blow flies (<i>Trypocalliphora braueri</i>)	15	13	11.5	3	20
Mites (<i>Dermanyssus</i> sp.)	8	100	521 \pm 183	28	1379

separately in paper bags for about two weeks. Each nest was then put in Berlese-Tullgren funnels (Southwood 1978, Clayton & Walther 1997) where arthropods were extracted for a period of 14 days and conserved in 70% ethanol. The extracted parasites were sorted and counted on a petri dish using a Leica Wild M8 stereo microscope. In total, five different groups of ectoparasites were found, of which the haematophagous hen fleas *Ceratophyllus gallinae* and mites of the genus *Dermanyssus* showed a prevalence of 100% each (Table 1). Mites were too numerous to be counted individually, and their abundance in each sample was therefore estimated by subsampling. Four subsamples were taken in circles with a diameter of 11.8 mm, out of a total sample in a petri dish with diameter 88.1 mm. We calculated the mean number of mites from the four subsamples and multiplied it with 55.74 (area of petri dish/area of a sample unit) to find an estimate of the total number of mites in each sample. We did not find any ectoparasites in nests fumigated after hatching in 1995 ($n = 11$), even though some of the inhabiting birds might have brought parasites with them which could have re-colonized the nests. This suggests that the experimental treatment was efficient. Although we cannot know if our ectoparasite counts reflected the situation also in the experimental nest boxes, this is likely since all boxes and nests used for the experiment in 1996 were selected from the same pool of boxes as those analysed for nest-dwelling ectoparasites.

RESULTS

One of the nest box pairs was occupied by a Wryneck *Jynx torquilla*, but the remaining 13 pairs were all used by Pied Flycatchers. These flycatcher pairs clearly selected the parasite-free nest box, as 12 pairs built nests in the fumigated box and only one pair chose the unmanipulated control box with ectoparasites present (Binomial test: $p < 0.004$).

DISCUSSION

This study shows that Pied Flycatchers prefer nest-sites that do not contain ectoparasites if they have a choice. This should not be surprising given the negative effects that nest-dwelling ectoparasites may have on both the parents and their offspring, as shown in a range of bird species (Møller et al. 1990, Løye & Carroll 1998, Fitze et al. 2004), including the Pied Flycatcher (Merino & Potti 1995a, Merino & Potti 1996, Potti et al. 1999). Nevertheless, our results contrast with a similar study from Sweden (Olsson & Allander 1995) where both flycatchers and three species of tits showed no preference for parasite-free nest boxes. The experimental design of the two studies was similar

(data from one year, same type of insecticide and timing of spraying), but the sample size of Olsson and Allander (1995) was twice that of ours, and two-thirds of the nest boxes they used contained old nests of tits (*Parus* sp.), not Pied Flycatchers. It is possible that the choice of nest boxes in the Swedish study was obscured by tit nests being included in the experiment, although the flycatchers did not show any preference for either of the two nest types. An alternative explanation for discrepancies between the two studies might be found in ambient conditions of the two study areas.

Prevalence of ectoparasites is known to vary with a range of environmental conditions which cause differences both within and between populations (Merino & Potti 1996, Rendell & Verbeek 1996, Heeb et al. 2000, Remeš & Krist 2005, Moreno et al. 2009). The most plausible explanation why our results differ from the earlier Swedish study may therefore be between-site differences in parasite numbers. Near Uppsala in Sweden, Olsson and Allander (1995) found on average 11 fleas in nest boxes where old nests were still present, while we recorded about five times more fleas in our study population in addition to several other parasitic arthropods (Table 1). Hence, the prevalence of ectoparasites in the Swedish study may have been too low to make it adaptive for flycatchers to respond to the presence of ectoparasites. This may not be a general situation within the area, though, as both the abundance and the relative severity of different groups of ectoparasites vary annually in relation to changing climatic conditions (Merino & Potti 1996). From this one might predict the existence of a threshold ectoparasite intensity above which it would be adaptive for birds to be choosy when selecting a nest site. Future studies should be conducted where the numbers of fleas in old nests are varied to see if such a threshold could be identified more directly.

Even if Pied Flycatchers prefer parasite-free nest sites, there are several reasons to assume that this choice is heavily constrained under natural circumstances. First, the supply of tree holes is typically limited in secondary cavity-nesters and competition for nest holes is high both within and between species (Newton 1994). Second, cavities may vary qualitatively in various traits which affect the choice of nest site. Pied Flycatchers are for example known to prefer upright nest boxes over tilted ones (Slagsvold 1986) and dry nest cavities over moist (Slagsvold & Lifjeld 1988). The position of a potential nest site in relation to habitat quality and distance to feeding areas is also important (Askenmo 1984, Siikamäki 1995). Moreover, whether the presence of an old nest in a cavity should be used as a positive or negative cue in nest site selection is debated. In Pied Flycatchers it has been suggested that old nests may be avoided in areas with a high prevalence of ectoparasites but not elsewhere (Merino & Potti 1995b). Cavities with old nests may even be

preferred over empty cavities if old nest material would indicate earlier successful breeding and low predation risk (Orell *et al.* 1993, Mappes *et al.* 1994, Olsson & Allander 1995), or because re-using old nest material results in a reduced workload during nest building (Loukola *et al.* 2014). In general, being choosy may incur costs to the selective bird in terms of time and energy invested and because of the risk of losing the best nest site to competing individuals (Oppliger *et al.* 1994, Olson & Allander 1995). Hence, in natural situations birds should be expected to assess a suite of qualitative traits, and balance these against each other and the time needed to search for better sites to find the most optimal nest site available. The present study clearly suggests that Pied Flycatchers are able to use the occurrence of ectoparasites in the nest cavity as a cue in this evaluation process, regardless of whether old nest material is present or not.

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